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Thiopental Monitoring by Gas-Chromatography

By *W. R. Külpmann, R. Fitzlaff*
Technical assistance: *K. Petry* and *L. Lütke-Holz*

Institut für Klinische Chemie,

A. Spring and *H. Dietz*

Neurochirurgische Klinik,
Medizinische Hochschule Hannover

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Summary: A gaschromatographic method is presented for the determination of thiopental (and of its metabolite pentobarbital) in cases of severe head injuries treated by induction of therapeutic barbiturate coma. To 1 ml serum the internal standard and saturated ammonium sulphate solution are added. The mixture is extracted by chloroform and the concentrated organic phase is injected into the gaschromatograph (stationary phase: SP 2510 DA). Imprecision from day to day: Coefficient of variation 7.7%; recovery 97%. The specificity was checked by comparison with the retention time of more than 80 drugs. One determination is accomplished within 1 hour.

Überwachung der Thiopentalbehandlung mittels gaschromatographischer Bestimmung der Konzentration im Serum

Zusammenfassung: Die Methode dient zur Bestimmung von Thiopental (und seines Metaboliten Pentobarbital) im Rahmen der Behandlung von Patienten mit Schädelhirntrauma mit hohen Dosen Thiopental.

1 ml Serum wird mit dem internen Standard und gesättigter Ammoniumsulfatlösung versetzt. Nach dem Mischen wird mit Chloroform extrahiert und die eingeengte organische Phase in den Gaschromatographen eingespritzt (stationäre Phase: SP 2510 DA). Präzision von Tag zu Tag: Variationskoeffizient 7,7%; Wiederfindung: 97%. Die Spezifität wurde geprüft durch Vergleich mit den Retentionsindices von über 80 Pharmaka. Eine Bestimmung dauert 1 Stunde.

Introduction

Barbiturate loading is of increasing importance in the treatment of neurosurgical patients (1–3). One of the favourite barbiturates used for this purpose is thiopental. To be sure that the therapeutic range is quickly achieved and maintained in these patients, who often additionally suffer from hepatic or renal failure, drug monitoring is necessary. For the routine use of this therapy a method is needed that is practicable and can easily be run round the clock. Because of its specificity gaschromatography is preferred for the determination of thiopental. But among the gaschromatographic methods published so far some are rather sophisticated as they are primarily adapted to pharmacokinetic studies, where very low concentrations have to be measured. They involve time consuming sample preparation, derivatization, and special detectors (alkali flame ionization detector, electron capture detector) (4–5) that are not available in many hospitals. Gaschromatographic methods using the widespread though less sensitive flame ionization need a time consuming sample preparation (6), derivatization (7) or column reconditioning after 6–12 samples in order to eliminate "dirt" peaks (8).

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A method is presented that is appropriate for the routine monitoring of thiopental in neurosurgery because of its practicability (simple clean up, no derivatization, use of flame ionization detector, no temperature programming) and reliability.

Materials and Methods

Thiopental sodium [5-ethyl-5-(1'-methyl-butyl)-2-thio-barbiturate sodium], M_r 264.4 (Byk Gulden Lomberg, Konstanz); Cyclobarbital-Ca [5-ethyl-5-(cyclohex-1'-en-yl) barbiturate-$Ca_{1/2}$], M_r 255.3 (Bayer, Leverkusen).
Disodium sulphate p.a., ammonium sulphate p.a., chloroform p.a. redistilled before use (Merck, Darmstadt).
Thiopental sodium is stabilised by the manufacturer by addition of 60 mg Na_2CO_3 to 1 g of the drug; 1151 mg of the mixture are dissolved in bidistilled water (4.13 mmol/l thiopental) and 230.2 mg/l in chloroform (0.83 mmol/l thiopental).
Cyclobarbital-Calcium is dissolved in bidistilled water (3.91 mmol/l); for gaschromatography the aqueous solution is extracted 3 times with chloroform, the organic phase is evaporated and made up with chloroform (final concentration 391 µmol/l).
The solutions are stable for at least 4 weeks at 4 °C.

Gaschromatography

Glass column: 1.8 m length; 2 mm internal diameter.
Stationary phase: GP 2% SP 2510 DA on Supelcoport 100/120 mesh (Supelco, Bellefonte, USA).
Gaschromatograph: Hewlett Packard 5880 A equipped with a flame ionization detector (Hewlett Packard, Frankfurt). Oven: 210 °C; inlet: 230 °C; detector: 240 °C. Carrier gas: Purged nitrogen 30 ml/min.

Procedure

To 1 ml serum 30 µl aqueous cyclobarbital solution and 2 ml saturated ammonium sulphate solution are added. The mixture is extracted with 5 ml chloroform, centrifuged 10 min at 3000 *g* and the separated organic phase is dried by passage through small columns filled with 2 g sodium sulphate. This procedure is repeated two times and the combined eluates are evaporated. The residue is dissolved in chloroform and injected into the gaschromatograph. The thiopental concentration of the samples is computed by means of the regression line of the peak heights of the standard solution.

Results

Precision in the series

Serum was spiked with an aqueous solution of thiopental and 1 ml aliquots were extracted as described. The coefficients of variation ranged from 4.2 to 5.9% (tab. 1).

Precision from day to day

Aliquots of spiked serum were frozen and thawed for analysis. The coefficient of variation was 7.7% for 10 single determinations (tab. 1).

Accuracy

Linearity was demonstrated from at least 41.3 to 371.3 µmol/l (fig. 1). The recovery without correction by the internal standard was 97% at a concentration of 41.3 µmol/l.

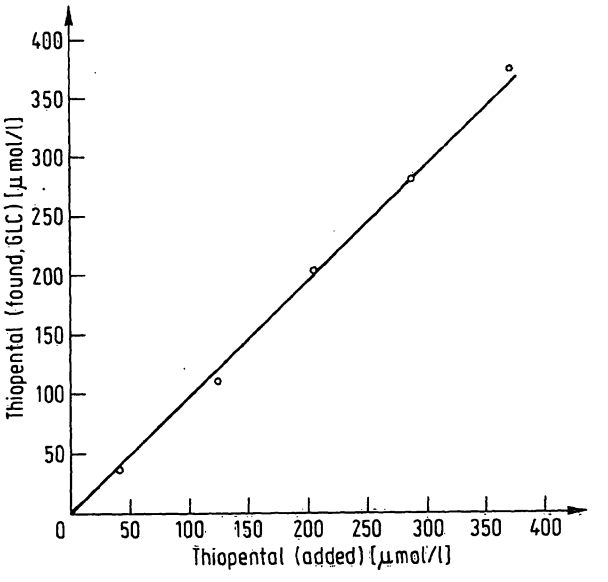

Fig. 1. Relationship between the concentration of thiopental added and thiopental as determined by gaschromatography.

Specificity

As a check for specificity the retention indices of the following drugs were determined according to *Kováts* (9). A complete list will be published separately (10). The most relevant retention indices are given in brackets.

Acetylsalicylic acid
allobarbital
amikazin sulphate
aminophenazone

Tab. 1. Determination of thiopental: Precision

	Number of determinations n	Target value (µmol/l)	Mean value $\bar{x}$ (µmol/l)	Deviation from the target value (%)	Coefficient of variation (%)
Precision in the series	10	41.3	40.1	−2.9	5.9
	10	206.3	210.0	+1.8	4.2
Precision from day to day	10	41.3	44.1	+6.8	7.7

amitriptyline
amobarbital
amphetamine sulphate
aprobarbital
barbital
benzydamine hydrochloride
brallobarbital
butalbital
carbamazepine
carbenicillin disodium
carbromal
chloramphenicol
chlordiazepoxide
chlorpromazine
chlorprothixene
clomethiazole ethanedisulphonate
codeine
crotarbital
cyclobarbital [3010]
cyclopal
desipramine hydrochloride
dextromoramide hydrogen tartrate
dextropropoxyphene hydrochloride
diazepam
2,2-diethylallylacetamide
diphenhydramine
doxepin hydrochloride [2715]
erythromycin
ethinamate
ethosuximide
flufenamic acid
glutethimide
guaiphenesin
haloperidol
heptabarbital
hexobarbital
imipramine hydrochloride
indomethacin
kanamycin sulphate
levorphanol tartrate
lidocaine hydrochloride
metamizol
methaqualone
methotrexate
methylphenobarbital
methyprylone
niflumic acid
nitrazepam
normethadone hydrochloride
oxazepam
oxyphenbutazone
paracetamol
pentobarbital [2665]
pethidine
phenacetin
phenobarbital
phenprocoumon
phenylbutazone
phenytoin
primidone
probenecid
procainamide hydrochloride
promethazine hydrochloride
propallylonal
propylhexedrine
pyrithyldione
quinidine sulphate
quinine dihydrochloride
salicylic acid
sebutabarbital
secobarbital
sisomycin sulphate
streptomycin sulphate
sulphadimethoxine
sulphamethoxydiazine
sulphisoxazole
tetracycline hydrochloride
theophylline
thiopental [2730]
thioridazine hydrochloride
tobramycin
tolbutamide
triflupromazine hydrochloride
trimethoprim
vinylbital.

Of all these substances, only doxepine can interfere with the quantitative determination: It is extracted in high yield (56%) by this procedure and has a retention index similar to thiopental. The therapeutic concentration of doxepine (0.04—0.72 μmol/l) (11) however is beyond the detectability of the method and a noticeable interference will only be encountered in lethal doxepine poisoning (35.80 μmol/l) (11). Endogenous serum constituents do not interfere with the determination (fig. 2).

As an estimate of detectability the precision of the method at low concentration was used. In serum spiked with 4.1 μmol/l thiopental, a concentration of 5.2 μmol/l and a coefficient of variation of 22.8% were found (n = 8). In thiopental therapy of head injury 124 to 289 μmol/l is considered as the (preliminary) therapeutic range (12) needed to achieve a burst suppression electroencephalogram; this is based on determinations of multiple samples taken from each of 15 patients during the course of their disease. The determination of a sample takes 1 hour after receipt by the laboratory.

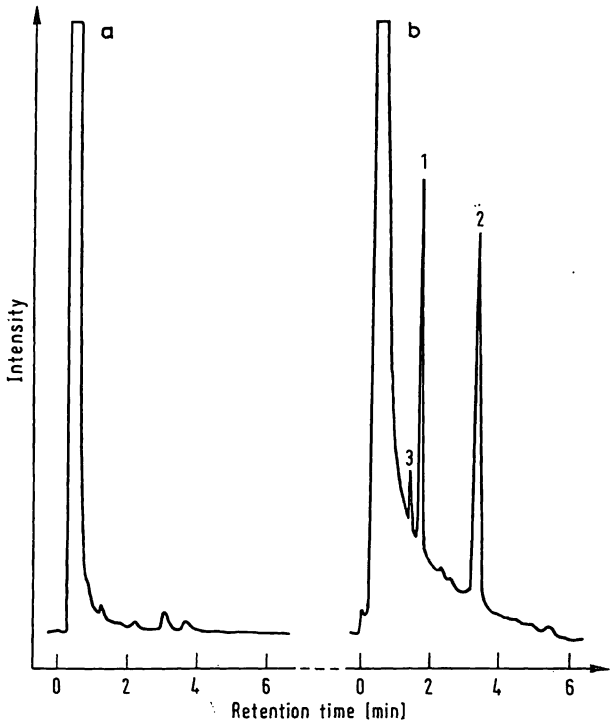

Fig. 2. a) Gaschromatogram of an extract of drug-free serum
b) Gaschromatogram of serum taken from a patient treated with thiopental
1 Thiopental (99.1 µmol/l)
2 Cyclobarbital (internal standard)
3 Pentobarbital (19.3 µmol/l)

Discussion

As shown above thiopental can be determined by this method without derivatization. Gaschromatography can therefore be run by use of an autosampler if many samples are to be analysed e.g. in pharmacokinetic studies, and a nitrogen sensitive detector can be used without precautions as well as a flame ionization detector. Alkylating procedures often give products that are unstable and must be injected immediately after addition of the reagent; otherwise the nitrogen containing derivatizing reagent leads to an overflow and to a contamination of the nitrogen flame ionization detector. It is a further advantage that pentobarbital as a hypnotic active metabolite of thiopental can be determined simultaneously. Especially in patients with reduced clearance rates the measurement of both thiopental and pentobarbital serum concentrations may prevent overdosage. Thiopental monitoring is a valuable method in high dose barbiturate therapy in addition to the EEG. Only by determination of the thiopental concentration one can decide if an isoelectric EEG is due to overdosage or to an irreparable breakdown of brain functions.

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Prof. Dr. W. R. Külpmann
Institut für Klinische Chemie
Medizinische Hochschule Hannover
Karl-Wiechert-Allee 9
D-3000 Hannover 61